S-ANTIGEN IMMUNOCYTOCHEMISTRY

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I. INTRODUCTION

The retina and the pineal complex of vertebrates share several common characteristics: 1) Both organs have developed as diencephalic evaginations (see von Frisch, 1911). 2) In poikilothermic vertebrates the pineal complex is endowed with sensory cells which display outer and inner segments and ultrastructurally resemble retinal photoreceptors (Eakin and Westfall, 1959; Oksche and von Harnack, 1963; Oksche, 1971; Collin, 1971). 3) Like retinal rods pineal photoreceptors contain, at least in their majority, immunoreactive opsin (Vigh and Vigh-Teichmann, 1981; Vigh- Teichmann *et al.*, 1982, 1983).

According to structural and ultrastructural criteria these similarities between the retina and the pineal organ are less conspicuous in mammals. Mammalian pinealocytes lack inner and outer segments typical of retinal and pineal photoreceptors. Therefore, in the literature, they have been regarded as entirely secretory (neuroendocrine) elements. However, the immunocytochemical demonstration of retinal S-antigen in the retina (Kalsow and Wacker, 1973) and in the pineal organ of the guinea pig (Kalsow and Wacker, 1977) indicates that mammalian pinealocytes have retained certain features of photoreceptor cells. Furthermore, immunization with retinal S-antigen induces not only "experimental autoimmune uveoretinitis" (Wacker and Lipton, 1968; Wacker and Kalsow, 1973; Wacker, 1981; Faure, 1980; Gery *et al.*, 1985), but also inflammatory reactions in the pineal organ (Kalsow and Wacker, 1978; Gery *et al.*, 1985).

These results must be viewed in context with the concept that, during evolution, pineal photoreceptors of poikilothermic vertebrates gradually changed into modified pineal photoreceptor cells of sauropsids and secretory pinealocytes of mammals (Oksche, 1965, 1971; Collin, 1971, Collin and Oksche, 1981).

S-antigen immunocytochemistry appears to be a valuable tool (i) to extend this concept to the molecular level and (ii) to establish similarities between the retina and the pineal organ on a representative comparative basis.

II. MATERIALS AND METHODS

A. Animals

1. Pisces, Teleostei
   Phoxinus phoxinus, *Salmo gairdneri*
2. Amphibia, Anura
   Rana temporaria, *Rana esculenta*, *Bufo bufo*
3. Reptilia, Lacertilia
   Lacerta sicula, *Tupinambis nigropunctatus*
4. Aves
   Passer domesticus, *Anas platyrhynchos*, *Coturnix coturnix*

5. Mammalia
   Didelphis virginiana, Erinaceus europaeus, Phodopus dsungorus, Mesocricetus auratus, Rattus norvegicus, Meriones unguiculatus, Oryctolagus cuniculus, Felis catus, Perodicticus potto, Lagothrix lagothrix, Aotes trivirgatus, Cebus, Macaca mulatta, Pongo pygmaeus, Homo sapiens

B. Tissue preparation

The animals were sacrificed either by perfusion of a fixative or by decapitation with subsequent immersion fixation of the dissected retinae and brains including the pineal organ. Human pineals were obtained from autopsies (the age of the subjects investigated varied from 2 days post partum to 89 years). Human retinae were obtained after surgical removal of one eye from 5 patients suffering from intraocular melanomas (2 patients) or perforating traumatization of the eye bulb (courtesy of Prof. W. Jacobi, Dept. of Ophthalmology, Giessen). Biopsy material of pineal tumors was provided by Prof. H.-W. Pia, Dept. of Neurosurgery, and Prof. W. Schachenmayr, Dept. of Neuropathology, Giessen. The following fixatives were applied: 1) SUSA fixative according to Heidenhain, 2) Bouin's fluid, 3) Carnoy's fluid, 4) 4% buffered paraformaldehyde, 5) 10 % formalin, 6) 2.5% buffered glutaraldehyde. Paraffin sections (5 to 7 µm thick) or frozen sections (25 µm thick) of the tissues were prepared for immunocytochemical analysis.

C. Immunocytochemical procedures

Immunoreactive S-antigen was detected by use of (i) a polyclonal and (ii) a monoclonal antibody against highly purified bovine S-antigen. The polyclonal antibody was raised in rabbits (for details, see Korf et al., 1985a). The monoclonal antibody was obtained by immunizing BALB/c mice with highly purified bovine retinal S-antigen and fusion of spleen cells with NS 1 myeloma cell lines. Binding of the antibodies was visualized by means of the peroxidase-antiperoxidase method of Sternberger (1979). Depending on the species and the fixation procedures, the dilution of the primary antibody varied from 1:400 to 1:4000. Immunocytochemical controls were performed by (i) replacing the polyclonal antibody with normal non-immune rabbit serum and the monoclonal antibody by ascites fluid of non-immunized mice, and (ii) incubating the sections with the diluted antibodies to which 100 nMol of purified bovine S-antigen (Zigler et al., 1984) was added.

III. RESULTS

All fixatives tested, with the exception of glutaraldehyde, were suitable for detection of S-antigen-immunoreactive material. Positive immunoreaction was observed in paraffin and frozen sections. However, the dilution of the antibody could be considerably increased (up to 1:4000) when frozen sections were used. On the other hand, the preservation of the structures was best in paraffin sections.

Generally, the polyclonal antiserum and the monoclonal antibody revealed similar results; however, the monoclonal antibody did not show immunoreactive S-antigen in the pineal organ of the opossum, Didelphis virginiana, and in retinae and pineal organs of all the primates examined, including man. Preabsorption of the primary antibodies with the purified antigen prevented the immunoreaction, thus indicating the specificity of the reaction.

Immunoreactive S-antigen exclusively occurred in the retina and the pineal organ. In contrast, other parts of the brain were immunonegative.

In the retina of the majority of species investigated the immunoreaction was restricted to the photoreceptor layer; other cells and layers were immunonegative (Fig. 1-4). However, in some species (e.g., the European minnow and the rat) positive immunoreaction was also found in individual cells scattered in the inner nuclear layer; these cells were very distinctly marked with the use of the monoclonal antibody. Particularly in primates some photoreceptor cells remained unlabeled (Fig. 3).

Immunoreactive S-antigen may occur in all portions of the retinal photoreceptor cells. Strong S-antigen immunoreaction was seen in the outer and inner segments. With interspecific variation immunoreactive S-antigen could also be visualized in the perikarya and the basal pedicles of these cells (Fig. 1, 3). In the human retina, S-antigen immunoreaction was primarily localized to outer segments of retinal photoreceptors (Fig. 4).

S-antigen-immunoreactive material was observed in the pineal organs of all species investigated with the exception of Passer domesticus, Aotes trivirgatus and Cebus.

In fishes and amphibians immunolabeled cells exhibited the characteristic features of true pineal photoreceptor cells. Immunoreactive outer and inner segments of these cells often protruded into the pineal lumen or the third ventricle (Figs.

5, 6). The intensity of the immunoreaction varied among individual cells; in the Carnoy-fixed material the immunoreaction was very strong in the perinuclear and supranuclear region of pineal photoreceptor cells (Fig. 6).

In the lacertilian and avian pineal organs immunoreactive cells resembled the "modified pineal photoreceptors (Fig. 7; for definition, see Collin and Oksche, 1981). Again, strongly immunoreactive cells were distinguished from cells displaying distinctly weaker immunoreaction or immunonegative elements. Occasionally, immunolabeled basal processes were traced toward the basal lamina enclosing the pineal follicles (Fig. 7).

In mammals the intensity of the immunoreaction and the number of immunoreactive cells differs considerably among the species. According to their size and shape, the immunoreactive cells in the mammalian pineal organ correspond to pinealocytes. In the opossum, very few immunoreactive pinealocytes were intermingled with immunonegative cells (Fig. 8). Several of the stained pinealocytes exhibited labeled processes directed toward the pineal lumen of the pineal recess of the third ventricle where they terminated with a bulbous swelling (Fig. 8).

The vast majority of pinealocytes was strongly immunoreactive in the cat (Fig. 9) and the rodents studied. In the latter, immunoreactive pinealocytes extended in rostral direction and were scattered in the medial habenular complex. S-antigen immunoreactive processes originating from pinealocytes in the deep portion of the pineal organ penetrated deeply into the medial habenular complex, the region of the posterior and the pretectal area. These processes were very conspicuous in the Djungarian hamster.

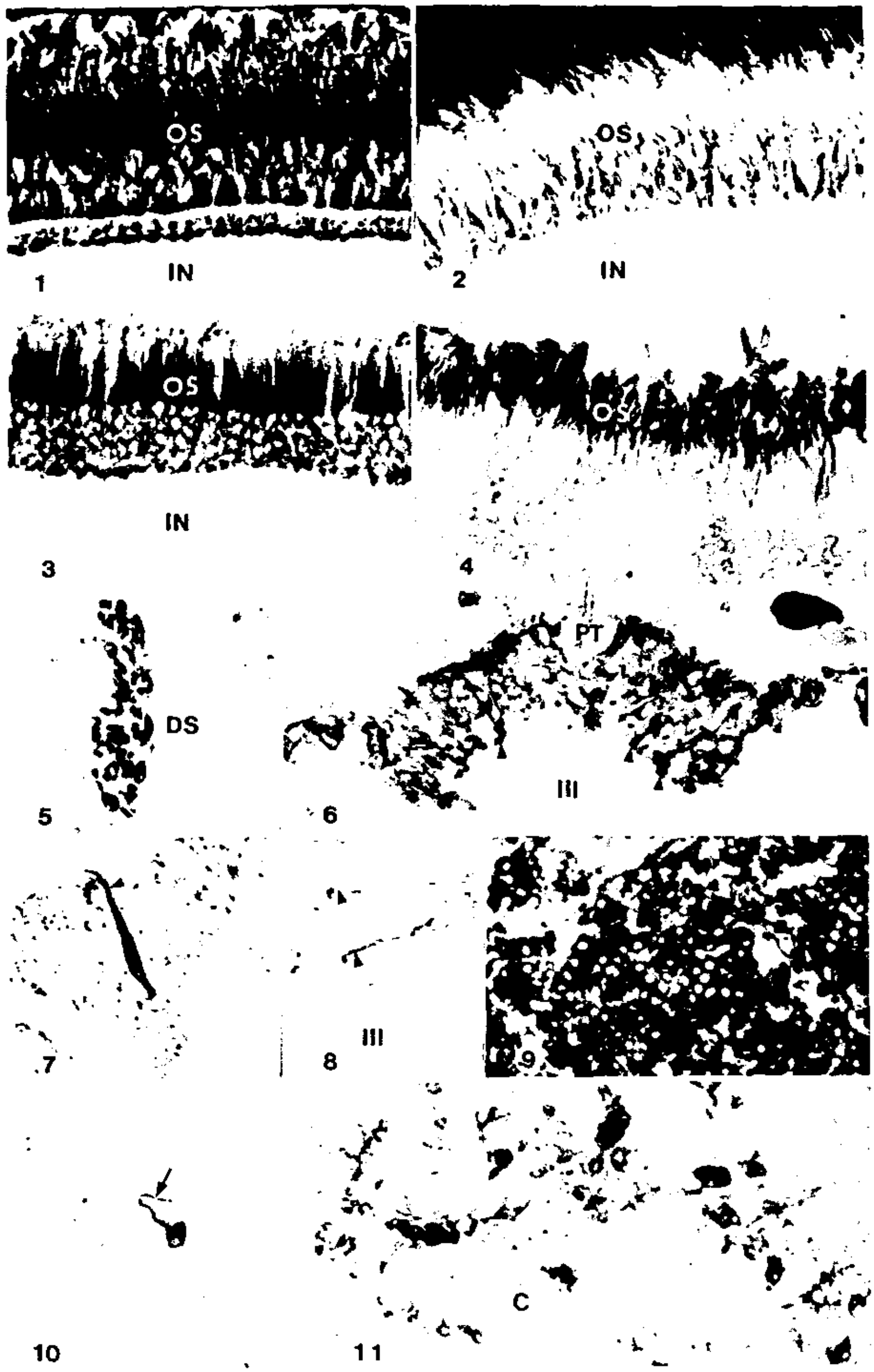
No immunoreactive cells were found in the pineal organs of <u>Aotes trivirgatus</u> and <u>Cebus</u>; other primates including man exhibited only very few labeled pinealocytes between non-immunoreactive elements (Fig. 10). Occasionally, processes of these cells were stained by the immunoreaction.

S-antigen immunocytochemistry was also applied to certain tumors of man (1 retinoblastoma, 1 pineocytoma, 1 pineoblastoma, four embryonic tumors of the pineal region). Immunoreactive neoplastic cells were present in the retinoblastoma and the pineocytoma (Fig. 11); the other tumors were completely devoid of immunoreaction.

IV. DISCUSSION

By means of immunocytochemistry, S-antigen has been demonstrated in a representative number of vertebrate species, including man. Positive immunoreactions were exclusively observed in the retina and the pineal organ. In contrast, other parts of the brain were immunonegative. Immunoreactive S-antigen was located in retinal photoreceptors and the different structural variants of the pinealocytes, i.e., pineal photoreceptors of poikilothermic vertebrates, modified pineal photoreceptors of sauropsids and pinealocytes of mammals (see also Kalsow and Wacker, 1973, 1977; Faure et al, 1984; Mirshahi et al., 1984; Korf et al., 1985a) These results are of significance for several reasons: 1) They indicate similarities between retinal photoreceptors and pinealocytes in all classes of vertebrates. 2) They support the concept that the different structural variants of pinealocytes belong to a common cell line of the "receptor type" (Collin, 1971; Oksche, 1971; Collin and Oksche, 1981). The selective expression of S-antigen in retinal photoreceptors and pinealocytes speaks in favor of the hypothesis that these cells are closely related in genetical terms and have developed from a common neuronal precursor probably located in the anlage of the diencephalon (see von Frisch, 1911).

Figs. 1-11. S-antigen immunocytochemistry. **1.** Phoxinus phoxinus, retina, frozen section; fixative: 4% paraformaldehyde (PF). **2.** Salmo gairdneri, retina, paraffin section; fixative: SUSA (S). **3.** Aotes trivirgatus, retina, paraffin section, fixative: Bouin's fluid (B). **4.** Human retina, paraffin section, fixative: 10% formalin (F). **5.** Phoxinus phoxinus, pineal organ, paraffin section, fixative: S. **6.** Rana esculenta, pineal organ, paraffin section, fixative: Carnoy's fluid (C). **7.** Tupinambis nigropunctatus, pineal organ, paraffin section, fixative: B. **8.** Didelphis virginiana, pineal organ, paraffin section, fixative: B. **9.** Felis catus, pineal organ, paraffin section, fixative: B. **10.** Macaca mulatta, pineal organ, paraffin section, fixative: B. **11.** Pineocytoma of a 49-year-old patient, paraffin section, fixative: F. OS outer segments, IN inner nuclear layer, DS dorsal sac, PT pineal tract, III third ventricle, C capillary, arrowheads pinealocyte processes directed toward the pineal lumen or third ventricle, arrow basal process of pinealocyte. **Figs. 1-4, 6, 9, 11** x280; **Figs. 5, 8** x180; **Figs. 7, 10** x320.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Immunoreactive S-antigen may occur in all portions of retinal photoreceptors and pinealocytes including their basal processes. As these processes are devoid of granular endoplasmic reticulum we assume that the S-antigen, like other proteins, is transported to the basal processes via axoplasmic flow.

The polyclonal antibody used in the present study allowed immunocytochemical demonstration of retinal S-antigen in all species investigated, including man. This suggests that several antigenetic determinants of the S-antigen molecule are well preserved during phylogeny and widely distributed in vertebrates. On the other hand, the opossum and all primates investigated including man apparently lack epitopes recognized by the monoclonal antibody. Our observations conform to findings of Faure et al. (1984) and Mirshahi et al. (1985) showing that the S-antigen is composed of species-specific and non-species-specific epitopes.Some of the non-species-specific epitopes were found even in photoreceptors of certain invertebrates (Mirshahi et al., 1985).

These findings indicate (i) the stability of the S-antigen molecule during phylogeny and (ii) its specific relation to photoreceptor cells and photoreceptive mechanisms. The precise functional role of the S-antigen has been a matter of dispute. Recent studies with the retina have provided evidence that the S-antigen is identical to the "48 K protein" (Pfister et al., 1984; Buzdygon et al., 1985). This protein responds to light signals by binding to rod outer segment membranes (Kühn, 1978, 1984); it mediates rhodopsin-catalyzed ATP-binding and quenching of cyclic GMP-PDE activation (Buzdygon et al., 1985).

The demonstration of a photoreceptor-specific protein in all types of pinealocytes is closely related to the functional role of these cells. It is well established that pineal photoreceptors of poikilotherms are capable of perceiving light stimuli and transmitting them to secondary intrapineal neurons by means of a synaptic mechanism (Dodt, 1973; see also Meissl, this volume). Modified pineal photoreceptors of sauropsids do not establish conspicuous synaptic contacts with intrapineal neurons; however, as shown by biochemical in-vitro experiments these cells have retained direct sensitivity to light and are capable of transformation of light stimuli into a neuroendocrine response (Deguchi, 1981). Thus, the modified pinealocytes may be classified as photoneuroendocrine cells (Oksche, 1971, 1983; see also Oksche and Hartwig, 1979) that respond to light stimuli by means of a neuroendocrine reaction at the cellular level. Mammalian pinealocytes have been considered as entirely secretory elements that have lost their photosensitivity. However, the immunocytochemical demonstration of the S-antigen in pinealocytes of numerous mammals may cast new light on the functional significance of this cell type.

Considering the the phylogenetic stability of the S-antigen and its specific relation to photoreceptor mechanisms one may conclude that this protein serves similar functions in pinealocytes. This might indicate that mammalian pinealocytes still possess characteristics of photoreceptor cells as is also suggested by the observations that (i) the pineal organ of the rat contains high amounts of rhodopsin kinase (Somers and Klein, 1985) and (ii) mammalian pinealocytes may also display other photoreceptor-specific markers such as immunoreactive Interphotoreceptor Binding Protein (IRBP) (Chader and Wiggert, this volume) and immunoreactive opsin (Korf et al., 1985b).

Two additional results obtained with S-antigen immunocytochemistry may provide further insight into the function ofmammalian pinealocytes. 1) In the atypical pineal organ of the opossum a number of immunoreactive pinealocytes displayed processes directed into the third ventricle or into the pineal recess. Such an intimate relationship between immunoreactive pinealocytes and the cerebrospinal fluid (CSF) is also present in the deep portion of the pineal organ of the hamster and the Mongolian gerbil (see also Korf et al., 1985a). When protruding into the pineal recess or the third ventricle these cells closely resemble S-antigen-immunoreactive pineal photoreceptors of poikilothermic vertebrates (compare Fig. 6 and 8) or, in more general terms, CSF-contacting neurons, which are regarded as receptor elements (Vigh-Teichmann and Vigh, 1983). This structural pattern may reflect a receptive capacity of certain pinealocytes (see also Quay, 1984). 2) It was very obvious in the Djungarian hamster that S-antigen-immunoreactive pinealocytes give rise to immunoreactive processes of beaded appearance, which leave the deep pineal organ and penetrate deeply into the medial habenular nucleus, the region of the posterior commissure and the pretectal area. This suggests that the mammalian pineal organ may not act exclusively via neuroendocrine mechanisms (i.e., by the release of melatonin), but also by means of direct projections into the brain. Also this finding suggests that mammalian pinealocytes have not completely lost neuronal features characteristic of retinal and pineal photoreceptor cells.

Interestingly, the number of S-antigen-immunoreactive pinealocytes varied among different species as did the intensity of the immunoreaction among individual pinealocytes. In rodents and in the cat most of the pinealocytes contained immunoreactive S-antigen; in primates and man, however, only a very limited number of pinealocytes displayed a positive immunoreaction. In man, proteolytic processes might account for a postmortal loss of the immunoreactivity. This possibility can be excluded for the non-human primates, which were fixed by perfusion. In further studies it must be established whether (i) these differences in immunostaining reflect different functional stages of a single cell type or (ii) they depend on the existence of two or more types of pinealocytes. In several mammalian species intensely and weakly stained pinealocytes are arranged in a peculiar topographical pattern. Similar patterns were also observed when the serotonin content of individual pinealocytes was investigated by use of the histofluorescence technique (cf. Vollrath, 1981). These findings may speak in favor of the existence of two or more types of pinealocytes.

Like in the pineal organ, intensely stained elements could be distinguished from immunonegative cells in the retina. Based on investigations of retinae of Tamias striatus and the rhesus monkey, Broekhuyse and Winkens (1985) suggested that immunoreactive S-antigen is restricted to retinal rods; however, in other vertebrate species immunoreactive S-antigen was found in retinal rods and cones (Faure et al., 1984; Mirshahi et al., 1984). Systematic immunocytochemical studies at the ultrastructural level are required to elucidate these differences in immunostaining among individual photoreceptor cells. These investigations may also help to clarify whether the S-antigen-immunoreactive elements scattered in the inner nuclear layer of the retina in some species represent displaced photoreceptors or Landolt's clubs.

Finally, the relevance of S-antigen immunocytochemistry for neuropathological investiagtions should be discussed. The finding that immunoreactive S-antigen is a selective marker of retinal photoreceptors and pinealocytes suggests that S-antigen immunocytochemistry may become a valuable tool in pathohistological investigations of brain tumors of man that are related to derivatives of a photoreceptor-producing primordium (i.e., the retina and the pineal organ).

Immunoreactive S-antigen occurs in certain neoplastic cells of retinoblastomas in the G0/G1 phase of the cell cycle (Donoso et al., 1985). We were able to confirm these results in our laboratory. Furthermore, we investigated several tumors of the pineal region (1 pineocytoma, 1 pineoblastoma, 1 germinoma II-III, 2 teratomas, 1 embryonic tumor). S-antigen-immunoreactive cells were exclusively found in the pineocytoma (Korf et al., 1985c). These preliminary results indicate that S-antigen immunocytochemistry may supplement conventional neuropathological investigations and, thus, help to characterize tumors in the pineal region of man more precisely.

Recently we have also started to analyze medulloblastomas, which occasionally resemble retinoblastomas in their histological features. Three tumors out of nine investigated to date contained S-antigen-immunoreactive cells. These results are of considerable importance for the classification of medulloblastomas; they may indicate that at least certain types of these tumors might have some kind of relationship to the photoreceptor-producing primordium of the brain. Studies are in progress to prove the validity of this hypothesis.

V. SUMMARY

The immunocytochemical demonstration of S-antigen reveals that this protein is exclusively located in retinal photoreceptors and pinealocytes of different vertebrates. This suggests that both cell types have developed from a common diencephalic precursor. The results support the concept that mammalian pinealocytes are derivatives of pineal photoreceptor cells of poikilothermic vertebrates. Furthermore, they indicate that mammalian pinealocytes still bear characteristics of photoreceptor cells. S-antigen immunocytochemistry may become a valuable tool in the neuropathological diagnosis of brain tumors of man related to the retina or the pineal organ (retinoblastomas, pineocytomas, medulloblastomas).

REFERENCES

Broekhuyse, R.M., and Winkens, H.J. (1985). Curr. Eye Res. 4, 703.

Buzdygon, B., Philp, N., Zigler, J.S., Gery, I., and Zuckerman, R. (1985). Invest. Ophthalmol. Vis. Sci. 26 (Suppl.) (in press).

Chader, G.J., and Wiggert, B. (1985). In "Pineal and Retinal Relationships" (D.C. Klein and P. O'Brien, eds) (in press) Academic Press, New York.
Collin, J.-P. (1971). In "The Pineal Gland" (G.E.W. Wolstenholme and J. Knight, eds.), p. 79. Churchill-Livingstone, London.
Collin, J.-P., and Oksche, A. (1981). In "The Pineal Gland. Anatomy and Biochemistry. Vol. 1" (R.J. Reiter, ed.), p. 27. CRC-Press, Boca Raton, Florida.
Deguchi, T. (1981). Nature 290, 706.
Dodt, E. (1973). In "Handbook of Sensory Physiology. Vol. VII/3B" (R. Jung, ed.), p. 113. Springer, Berlin.
Donoso, L.A., Felberg, N.T., Augsburger, J.J., and Shields, J.A. (1985). Invest. Ophthalmol. Vis. Sci. 26, 568.
Eakin, R.M., and Westfall, J.A. (1959). J. biophys. biochem. Cytol. 6, 133.
Faure, J.-P. (1980). Curr. Top. Eye Res. 2, 215.
Faure, J.-P., Mirshahi, M., Dorey, C., Thillaye, B., de Kozak, Y., and Boucheix, C. (1984). Cuur. Eye Res. 3, 867.
Frisch, K. von (1911). Pfluegers Arch. ges. Physiol. 138, 319
Gery, I., Mochizuki, M., and Nussenblatt, R.B. (1985). Prog. Retinal Res. 5
Kalsow, C.M., and Wacker, W.B. (1973). Int. Arch. Allergy 44, 11.
Kalsow, C.M., and Wacker, W.B. (1977). Invest. Ophthalmol. Visual Sci. 16, 181.
Kalsow, C.M., and Wacker, W.B. (1978). Invest. Ophthalmol. Visual Sci. 17, 774.
Korf, H.-W., Møller, M., Gery, I., Zigler, J.S., and Klein, D.C. (1985a). Cell Tissue Res. 239, 81.
Korf, H.-W., Foster, R.G., Ekström, P., and Schalken, J.J. (1985b). Cell Tissue Res. (in press).
Korf, H.-W., Klein, D.C., Zigler, J.S., Gery, I., and Schachenmayr, W. (1985c). Acta neuropath. (in press).
Kühn, H. (1978). Biochemistry 17, 4389.
Kühn, H. (1984). Prog. Retinal Res. 3, 123.
Meissl, H. (1985) In"Pineal and Retinal Relationships" (D.C. Klein and P. O'Brien, eds.) (in press). Academic Press, New York.
Mirshahi, M., Faure, J.-P., Brisson, P., Falcon, J., Guerlotte, J., and Collin, J.-P. (1984). Biol. Cell 52, 195.
Mirshahi, M., Boucheix, C., Collenot, G., Thillaye, B., and Faure, J.-P. (1985). Invest. Ophthalmol. Visual Sci. 26, 1016.

Oksche, A. (1965). Prog. Brain Res. 10, 3.
Oksche, A. (1971). In "The Pineal Gland" (G.E.W. Wolstenholme and J. Knight, eds.), p. 127. Churchill-Livingstone, London.
Oksche, A. (1983). In "The Pineal Gland and Its Endocrine Role" (J. Axelrod, F. Fraschini, and G.P. Velo, eds.), p. 15. Plenum Press, New York. Oksche, A., and von
Harnack, M. (1963). Z. Zellforsch. 59, 582.
Oksche, A., and Hartwig, H.G. (1979). Prog. Brain Res. 52,113.
Pfister, C., Dorey, C., Vadot, R., Mirshahi, M., Deterre, P., Chabre, M., and Faure, J.-P. (1984). C.R. Acad. Sci.Paris 299, 261.
Quay, W.B. (1984). Pineal Res. Rev. 2, 87.
Somers, R.L., and Klein, D.C. (1985). Science 226, 182.
Sternberger, L. (1979). "Immunocytochemistry". J.Wiley-Sons, New York.
Vigh, B., and Vigh-Teichmann, I. (1981). Cell Tissue Res. 221, 451.
Vigh-Teichmann, I., and Vigh, B. (1983). Arch. histol. japon. 46, 427.
Vigh-Teichmann, I., Korf, H.-W., Oksche, A., and Vigh, B. (1982). Cell Tissue Res. 227, 351.
Vigh-Teichmann, I., Korf, H.-W., Nürnberger, F., Oksche, A., Vigh, B., and Olsson, R. (1983). Cell Tissue Res. 230,289.
Vollrath, L. (1981). In "Handbuch der mikroskopischen Anatomie des Menschen. Vol.IV/10" (A. Oksche and L. Vollrath,eds.), p. 1. Springer, Berlin.
Wacker, W.B. (1981). In "Proceeding Immunology of the Eye, Workshop II" (R.J. Helmsen, A. Suran, I. Gery, and R.B. Nussenblatt, eds.), p. 11. Information Retrieval Inc., Washington, D.C.
Wacker, W.B., and Lipton, M.M. (1968). J. Immunol. 101, 151.
Wacker, W.B., and Kalsow, C.M. (1973). Int. Arch. Allergy Appl. Immunol. 45, 582.
Zigler, J.S., Mochizuki, M., Kuwabara, T., and Gery, I. (1984) Invest. Ophthalmol. Visual Sci. 259, 77.

Acknowledgments. We are greatly indebted to Miss G. Döll for effective technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft (Ko 758/2-4).